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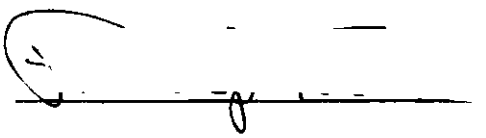

7/25/68

ATTEMPTED RECONSTITUTION OF DIHYDROSTREPTOMYCIN

A THESIS

Presented To

The Faculty of the Graduate Division

by

Phillip A. Torline

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

In the School of Chemistry

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ATTEMPTED RECONSTITUTION OF DIHYDROSTREPTOMYCIN

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GLOSSARY OF ABBREVIATIONS

BAW	t-Butyl alcohol:acetic acid:water, 2:1:1 (v/v), paper chromatography solvent system.
Cbz	Carbobenzoxy group
C.T.	Column temperature (GC).
GC	Gas chromatography.
R.T.	Retention time (GC).
SE-30	Silacone GC column liquid phase.
T.L.C.	Thin-layer chromatography.
T.M.S.	Trimethylsilane derivative.

SUMMARY

The purposes of this research were to determine (1) the required protecting groups for the reconstitution of dihydrostreptomycin, (2) the conditions necessary for their removal, (3) the preparation of the protected dihydrostreptobiosamine and streptidine moieties, and (4) the glycosidic union of these two moieties.

It was determined early in the research that an N-acetyl or N-benzoyl group of the N-methyl-L-glucosamine fragment could not be removed under mild conditions. Selective blocking of this amino function was achieved by the use of carbobenzoxy chloride. Dihydrostreptomycin was successfully regenerated from perbenzoyl-N-carbobenzoxydihydrostreptomycin by treatment of the latter with ammonia in methanol to remove the benzoyl groups followed by reduction to remove the carbobenzoxy group.

Both heptaacetyl- and heptabenzoylstreptidine are reported in the literature; however, only heptabenzoylstreptidine could be prepared pure. The preparation of the dihydrostreptobiosamine moiety began with the known ethyl thiodihydrostreptobiosaminide hydrochloride which by carbobenzoxylation and benzoylation yielded ethyl tetra-O-benzoyl-N-carbobenzoxythiodihydrostreptobiosaminide. Reaction of this compound with bromine and methanol or cyclohexanol in the presence of silver ion gave the corresponding methyl or cyclohexyl glycosides in good yield (70%). The configuration of the glycosides were tentatively assigned β by a comparison of their optical rotations with those of known streptobiosamine derivatives. All attempts to react ethyl tetra-O-benzoyl-N-carbobenzoxy-α-

thiodihydrostreptobiosaminide with heptabenzoylstreptidine or crude heptaacetylstreptidine failed.

CHAPTER I

INTRODUCTION

Dihydrostreptomycin

In 1944 a crude concentrate of an antibiotic prepared from the cultures of Streptomyces griseus was obtained that proved strongly bacteriostatic against gram positive and gram negative organisms (1). The broad spectrum of this antibiotic, called streptomycin, coupled with its low toxicity led to extensive study of its chemistry and pharmacology (2). The early literature on the isolation and development of streptomycin has been exhaustly reviewed (1).

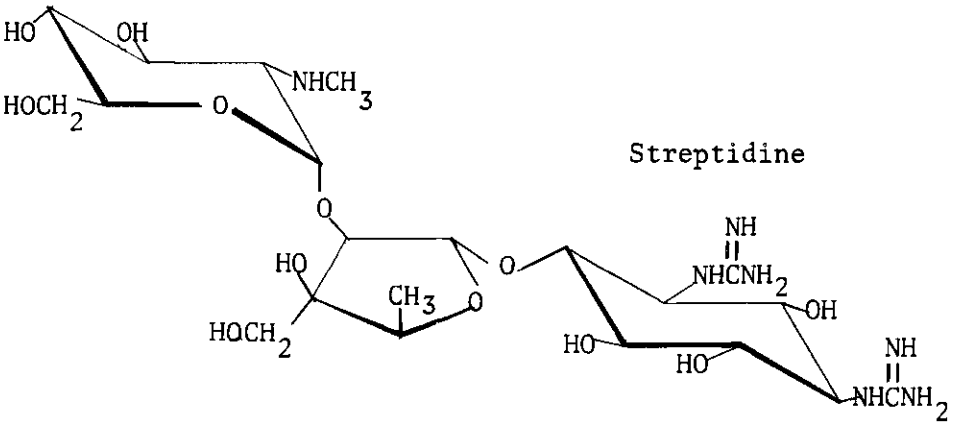
Hydrogenation of streptomycin at atmospheric pressure results in the consumption of one equivalent of hydrogen. The product, dihydrostreptomycin, differs only slightly from that of streptomycin in its potency and toxicity against microorganisms. The retention of biological activity in dihydrostreptomycin after such a chemical change is rather unique among the chemistry of antibiotics (2,3). More recently dihydrostreptomycin has been isolated from the fermentation cultures of Streptomyces humidus (4).

Structure of Dihydrostreptomycin

The chemistry of streptomycin and dihydrostreptomycin has been fully described (2,5,6). Dihydrostreptomycin is a water-soluble, thermostable, levoratotory, basic compound of molecular formula $C_{21}H_{41}N_7O_{12}$ (2). The molecule consists of the three fragments: N-methyl-L-glucosamine, L-

dihydrostreptose, and streptidine. The fragment N-methyl-L-glucosamine is glycosidically linked through C_1 to C_2 of dihydrostreptose to form the compound dihydrostreptobiosamine. The glycosidic linkage from C_1 of dihydrostreptose in dihydrostreptobiosamine to C_4 of streptidine yields dihydrostreptomycin. The structure and absolute configuration of dihydrostreptomycin is shown by structure I.

Dihydrostreptobiosamine



L-Dihydrostreptose

I

Dihydrostreptomycin

The structures and stereochemistry of all three moieties of dihydrostreptomycin have been confirmed by synthesis (5,6,7,8,9). The glycosidic unions of dihydrostreptomycin are the only remaining structural features to be defined by synthesis.

The assignment of the anomeric configuration in streptomycin and dihydrostreptomycin was not firmly established until 1966 (10). Wolfrom et al. (11) first reported a study on the configurations in dihydrostreptomycin. Their conclusions were based on the application of Hudson's rules of isorotation (12) and the assumption that the streptidine moiety was attached to streptose in such a manner that it made no contribution to the optical activity of dihydrostreptomycin. Thus, from the molecular rotation of dodecaacetyldihydrostreptomycin (11) ($[\alpha]_D$ - 67°), [M] = A_S + B = -72,900 (A_S is the rotatory contribution of the dihydrostreptose glycosidic carbon in the streptidine-streptose linkage, and B is the contribution of the remainder of the molecule) and the known anomeric forms of methyl pentaacetyldihydro-L-streptobiosaminide (13) (A' + B = -65,900° and -A' + B = -19,100), the value B(-42,500°) and A_S (-30,400) may be evaluated. The compound tetraacetyldideoxydihydro-L-streptobiosamine (14,15) gave [M'] = Ag + B' + X = -40,900, where Ag is the rotatory contribution of C-1 of the acetylated N-methyl-L-glucosamine, B' that of the remainder of the glucosamine portion, and X that of the optically active dideoxyldihydrostreptose moiety. The value of X was assumed to be approximately that of dideoxydihydrostreptose (4200) (16), and the term B' (-24,150°) was evaluated from the rotations (A' + B' = -41,600 and -A'' + B' = -6,700) of the known anomeric forms of pentaacetyl N-methyl-L-glucosamine (17), giving a value of Ag = -20,950. The numerical sign of the

values of Ag and A_S along with their magnitude was that expected if both the glycosidic linkages in dihydrostreptomycin were α-L.

However, it was later determined (18) that the streptidine moiety was asymmetrically attached to the dihydrostreptose moiety, making the streptidine moiety optically active by substitution. This fact required Wolfrom (19) to make a revision in his earlier calculation for the dihydrostreptose streptidine linkage.

The molecular rotation of dodecabenzoyldihydrostreptomycin (19), [M] = +126,500, was considered the sum of the component parts S, A_S, and B, where S was considered to approximate the molecular rotation of heptabenzoylstreptidine (18) ([M] = +57,500), A_S is the rotatory contribution of the dihydrostreptose glycosidic carbon in the streptidine dihydrostreptose linkage, and B that of the remainder of the benzoylated dihydrostreptomycin. From these values, therefore, $(A_S + B) = +69,000$. Since the compound methyl pentabenzoyldihydrostreptobiosaminide (19) shows $[M] = A_S' + B = 874\ (-10°) = -8,740$ which is the α-L form by the Hudson classification (12), the linkage between dihydrostreptose and streptidine was considered to be β-L.

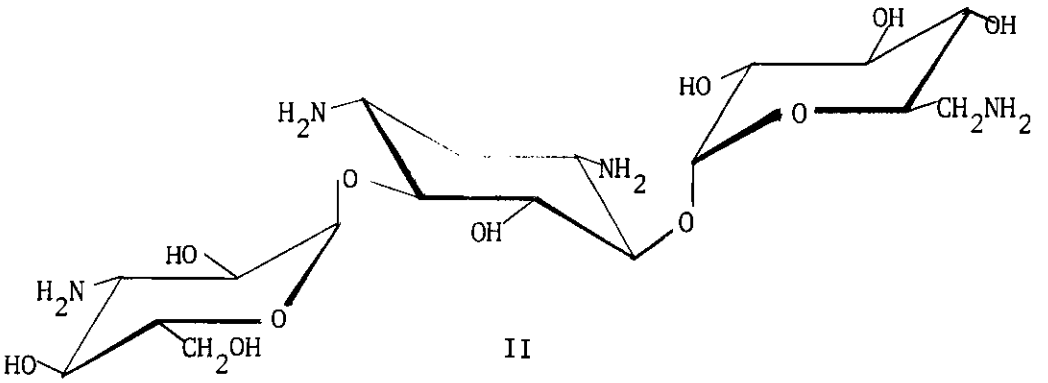
In 1966, Rinehart (10) published an n.m.r. study of dihydrostreptomycin. The coupling constant ($J \sim 3.0$ cps) of the anomeric proton of the N-methyl-L-glucosamine moiety corresponds to an axial-equatorial H-1, H-2 relationship, and thus the α-L-configuration for the N-methyl-L-glucosamine-dihydrostreptose glycosidic bond was indicated. However, the anomeric proton of dihydrostreptose occurs as a broad singlet $J \leq 1$ cps, which can only be the case when the H'-1, H'-2 relationship of a furanoside is *trans*. Since dihydrostreptose has the L-lyxo configuration

(7,20) an H'-1, H'-2 trans relationship corresponds to the α-L configuration for the dihydrostreptose-streptidine glycosidic bond.

All the configurational details of dihydrostreptomycin (relative and absolute) have recently been unequivocally determined by means of an X-ray study of the oxime selenate of streptomycin (21), and the assignment of the α-L configuration for both the glycosidic linkages was found to be correct.

The presence of α-glycosidic bonds in dihydrostreptomycin greatly increases the difficulty of achieving this structural feature by synthesis. The synthesis of β-pyranosides is readily achieved by the use of the Koenigs and Knorr reaction (22) in which an α-pyranosyl halide is reacted with a hydroxylic compound in the presence of a silver oxide. Application of this method to the synthesis of α-glycosides has been of little value because the β-pyranosyl halides are unstable and readily isomerized to the more stable α-isomer. Also, they usually react by a mechanism involving neighboring group participating by a protecting group at C-2. The result of this participation is a double displacement at C-1 and thus the β-configuration results. Various "modified" Koenigs and Knorr reactions have been attempted where a nonparticipating group was placed at C-2 (23), a special base was used (24,25), or a suitable combination of solvent and base was found that results in the formation of the α-linkage (25).

A recent example of a highly successful application of a "modified" Koenigs and Knorr reaction was the synthesis of kanamycin A (26), structure II, in which 6-amino-6-deoxy-D-glucose and 3-amino-3-deoxy-D-glucose are linked in the α-form at C-4 and C-6 positions of the 2-deoxystreptamine moiety, respectively.



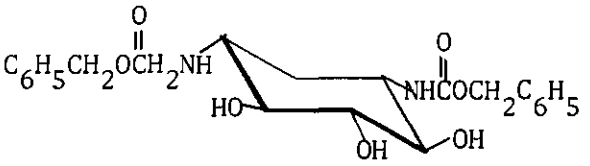
II

Kanamycin A

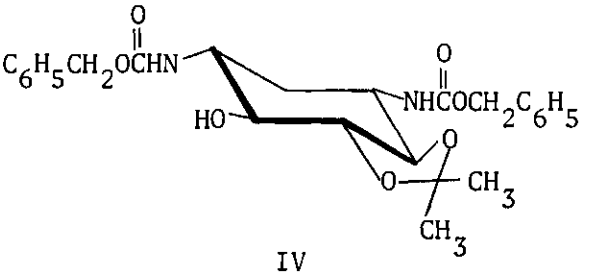
The initial step in the synthesis was the treatment of N,N'-dicarbobenzoxy-2-deoxystreptamine, III, with 2,2-dimethoxypropane to give the derivative IV (racemic form). The condensation of IV with the perbenzyl derivative of 6-acetamido-6-deoxy-D-glucopyranosyl chloride, V, by a "modified" Koenigs and Knorr reaction and the subsequent removal of the isopropylidene group gave the two isomers VIa and VIb in 74% yield. From the coupling constant and chemical shift of the proton at C-1, the α-configuration was assigned for these isomers. The reactions of VIa with 3-acetamido-2,4,6-tri-O-benzyl-3-deoxy-D-glucopyranosyl chloride, VII, by a second "modified" Koenigs and Knorr reaction gave the two isomers VIIIa and VIIIb in 55% yield. Removal of the protecting groups from VIIIa gave a material that was identical with an authentic sample of kanamycin A. The complete reaction sequence is shown in scheme I.

Possibly the most acceptable method of preparing α-glycosides has been the anomerization of β-glycosides with acidic catalysts. Boron trifluoride (27), stannic chloride (28), titanium tetrachloride (29), and

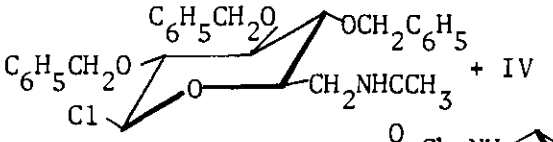
SCHEME I



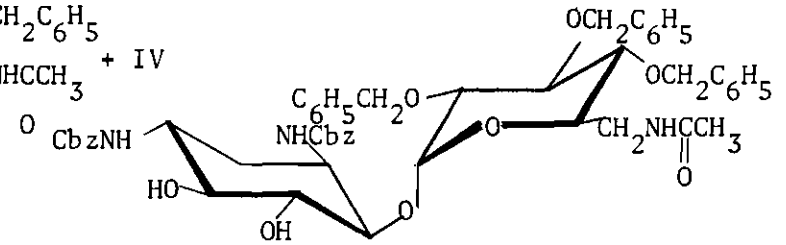
III



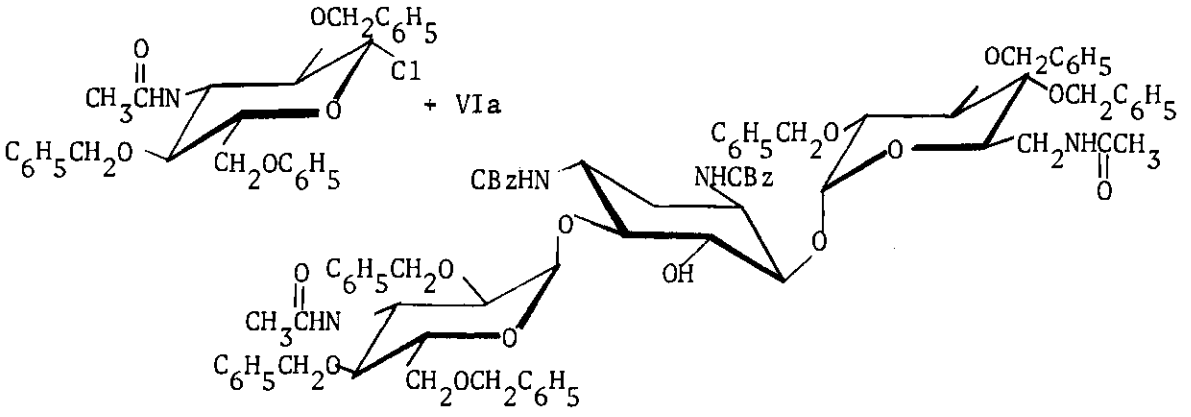
IV



V



VI a(α) + VIb(β)



VIIIA(α) + VIIIB(β)

VIIIa $\xrightarrow[2)\ Ba(OH)_2]{1)\ Na,\ liquid\ NH_3}$ II

sulfuric acid-acetic anhydride (27) has been found effective acid catalysts. Stenlake (25) attempted to isomerize the erroneously reported β-linkage between dihydrostreptose and streptidine with a variety of acid reagents, but since this linkage was already α, these efforts failed.

A less common method of obtaining glycosides has been with the use of thioglycosides. These compounds have not found wide application in synthetic carbohydrate chemistry primarily because of the difficulty of preparation. Contrary to alkyl glycosides, thioalkyl furanosides rather than thioalkyl pyranosides, are usually obtained. However, these compounds have been shown to react with inversion of configuration, and, hence, with a proper β-thioglycoside one may obtain α-glycosides in good yields (30).

Purposes of the Research

All the structural features of dihydrosteptomycin have been achieved by synthesis except the glycosidic bonds. Stenlake (25) has reported the synthesis, in low yields, of a number of streptidine-β-hexosides. No attempt to link streptidine glycosidically with dihydrostreptobiosamine has been reported. The purpose of this research was the preparation of a suitable dihydrostreptobiosamine derivative and to link it glycosidically with a protected streptidine moiety. Reaction conditions were to be sought that would yield the α-configuration. Removal of the protecting groups would then yield dihydrostreptomycin.

The reconstitution of dihydrostreptomycin will complete a major portion of the total synthesis of dihydrostreptomycin and aid in the synthesis of related compounds.

CHAPTER II

EXPERIMENTAL

Apparatus and Techniques

Anhydrous methanol was prepared as described elsewhere (5). Anhydrous pyridine was prepared by distillation from potassium hydroxide pellets and stored over potassium hydroxide pellets. Anhydrous chloroform was prepared just before use by washing it with distilled water followed by distillation from calcium chloride. Anhydrous carbon tetrachloride was prepared by distillation from phosphorous pentoxide powder. Anhydrous cyclohexanol was prepared by distillation from sodium metal. Anhydrous ether was purchased (Merck reagent 71633) and stored over sodium ribbon.

Concentrations and evaporations were performed using a modified Rinco (Model Ve-1000-A) rotating evaporator. The anhydrous magnesium sulfate (Mallinckrodt AR 6070) used to dry organic extracts and solution was always removed by gravity filtration and washed thoroughly with fresh solvent.

Melting points were observed using a Köfler hot stage. Microanalyses were performed by Bernardt Laboratories (Mülheim, West Germany). Infrared spectra were recorded using Perkin Elmer models 137 and 457 recording spectrophotometers. Potassium bromide was used for all pellet spectra. A Bellingham and Stanley polarimeter (Model No. 397619) was used to determine all optical rotations. The source of the sodium D line

was a General Electric Sodium Lab-Arc lamp. The error given in the optical rotations indicates the maximum deviation from the average value of five measurements.

All nuclear magnetic resonance (n.m.r.) spectra were determined at ambient temperature using Varian Model A-60 and A-60D spectrometers. Chemical shift values are reported in τ units ($\tau = 10 - \delta$), and either tetramethylsilane (TMS) or 2,2-dimethyl-2-silapentane-5-sulfonate (DDS) were used as internal standards. Spin-spin coupling constants, (J, measured in cps) given with more than one significant figure were determined using the 50 cps sweep width. Concentrations given for solution spectra are per cent by weight.

Silicic acid chromatography columns were prepared as described elsewhere (5). The column dimensions are given in the text. Thin-layer chromatography (TLC) was performed as previously described (5).

The regeneration and method of use of ion exchange resins were as described elsewhere (5). Resins were measured for use by allowing them to settle to constant volume in a graduated cylinder. All pH measurements were made using Hydrion paper (Micro Essential Laboratory).

Gas-liquid chromatography was performed using a Glowall Corp. Chroma-lab Model A-110 instrument equipped with a Minneapolis Honeywell continuous recorder. The columns employed were prepared and equilibrated as described elsewhere(5).

Trimethylsilane derivatives were prepared as previously described (6).

N-Carbobenzoxydihydrostreptomycin

Dihydrostreptomycin sulfate (5 g, 0.00685 mole) was dissolved in distilled water (30 ml), and the pH of the solution was adjusted to 10 by the addition of anion exchange resin (Amberlite, IR-400 (OH^-)) in the hydroxyl phase. Carbobenzoxychloride (10 ml, 90% by n.m.r. analysis, 0.0518 mole) was slowly added while the solution was stirred. After an additional 3 hr of stirring, the resin was removed by filtration and the filtrate was extracted with ether (3 x 30 ml); the ether was discarded. The aqueous solution was concentrated *in vacuo* at 50° to a volume of 5 ml, and the concentrate was passed over a column (30 ml, column dimensions: 15 cm x 1 cm) of anion exchange resin (Amberlite, IR-45 ($SO_4^=$)). A total of 50 ml of elutant was collected and lyopholyzed to yield N-carbobenzoxydihydrostreptomycin sulfate (4.68 g, 85%) as a white, amorphous solid.

Undeca-O-benzoyl-N-carbobenzoxydihydrostreptomycin

N-Carbobenzoxydihydrostreptomycin sulfate (5 g, 0.00615 mole) was benzoylated in the same manner as dihydrostreptomycin sulfate (35). The isolation of the product had to be modified slightly because of the ease of hydrolysis of the carbobenzoxy group. After the benzoylation was completed, chloroform (100 ml) was added, and the resulting solution was extracted with a saturated solution of cold sodium bicarbonate until all the excess benzoyl chloride had been hydrolyzed and the resulting benzoic acid neutralized. The soltuion was dried over magnesium sulfate, the magnesium sulfate removed by filtration, and the dry solution evaporated *in vacuo* at 50°. The resulting oil was dissolved in benzene and preci-

itated using petroleum ether (30-60°) as before to yield undeca-O-benzoyl-N-carbobenzoxydihydrostreptomycin (8.9 g, 79% as a crude, yellow, amorphous solid. No further purification was performed on this compound.

Regeneration of Dihydrostreptomycin Sulfate From Undeca-O-benzoyl-N-carbobenzoxydihydrostreptomycin

A mixture of undeca-O-benzoyl-N-carbobenzoxydihydrostreptomycin (2.0 g, 0.00108 mole) and dry methanol (50 ml) was cooled in an ice bath. Dry ammonia was passed into the mixture until the methanol was saturated. The reaction mixture was sealed and allowed to stand at room temperature for three days. After this period the methanol/ammonia was removed *in vacuo* at 50°. The resulting semi-crystalline mass was triturated with dry ether (3 x 10 ml); the ether was discarded. The resulting yellow, amorphous solid (0.75 g, 84%) was dissolved in dry methanol (75 ml), and the solution was placed under two pounds of hydrogen gas in the presence of 5% platinum on carbon (0.3 g) for 12 hr. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* at 50°. The residue was dissolved in distilled water (2 ml) and passed over a column (30 ml, column dimensions: 15 cm x 1 cm) of anion exchange resin [Amberlite, IR-45 ($SO_4^=$)]. A total of 80 ml of elutant was collected and lyopholyzed. The product (0.42 g, 55%) showed two Weber positive spots by paper chromatography according to Hedig (31) (R_F 0.00 and 0.78; an authentic sample of dihydrostreptomycin sulfate showed R_F 0.78).

The material was further purified by chromatography over Sephadex G-15 (180 ml, column dimensions: 150 cm x 1.5 cm) prepared in 0.01 N

formic acid. The material was eluted in fractions 8-13 (fraction volume; 20 ml; eluting solvent: 0.01 N formic acid) to yield a total of 350 mg (46%) of material. The n.m.r. spectrum of this material (R_F 0.78) was identical with that of authentic dihydrostreptomycin sulfate.

The trihelianthate salt of the material was prepared (32,33) and the salt was found to have an identical infrared spectrum with that of the authentic trihelianthate salt of dihydrostreptomycin.

Dodecaacetyldihydrostreptomycin

Acetic anhydride (1000 ml, 10.5 moles) was added in 50-ml portions over a period of 200 min to a stirred solution of streptomycin sulfate (Charles Pfizer and Co., 102 g, 0.143 mole) in methanol (2000 ml) and pyridine (600 ml). Anhydrous sodium acetate (104 g, 1.27 moles) was added, and the mixture was stirred for 24 hr. The mixture was then filtered by suction to remove sodium sulfate, and the filtrate was concentrated in vacuo at 50°. The resulting semi-solid mass was triturated with dry ether; the ether was discarded. The solid mass was treated with pyridine (1000 ml), and acetic anhydride (1000 ml, 10.5 moles) was added in 100-ml portions over a period of 100 min. The resulting solution was maintained at room temperature for 48 hr. After the solution was heated at 50° for an additional 5 hr, the solvents were removed in vacuo at 50°. The resulting oil was triturated with benzene (300 ml), and the mixture was filtered by gravity. The benzene filtrate was slowly added to vigorously stirred petroleum ether (1500 ml, b.p. 30-60°) to yield, after suction filtration, dodecaacetyldihydrostreptomycin (138 g, 0.129 mole, 90%) as a light yellow, amorphous solid. Reprecipitation of a small portion from benzene-petroleum ether (30-60°) gave a colorless solid that

showed at least four components by thin layer chromatography (R_F: 0.00, 0.04, 0.36, 0.47; developing solvent: 5% methanol/chloroform), $[\alpha]_D$ -68.4 ± 2.0 (c 1.0 chloroform), Lit. $[\alpha]_D$ -67.6 (34). No further purification was attempted.

Attempted Preparation of Heptaacetylstreptidine

A solution of dodecaacetyldihydrostreptomycin (2.1 g, 0.00215 mole) in absolute chloroform (30 ml) was treated with hydrogen bromide in acetic acid (31%, 1.8 ml) according to the procedure of Stenlake (25). The amorphous heptaacetylstreptidine dihydrobromide (0.69 g, 45%) showed a melting point of 193-200° [Lit. 180-189° (25)] and $[\alpha]_D$ - 3.3 ± 2° (c 3.0 methanol) [Lit. $[\alpha]_D$ -5.4 (c 3.0 methanol) (25)]. All attempts to prepare the free base of heptaacetylstreptidine according to the procedure of Stenlake (25) failed to yield a homogeneous material that gave a negative reaction with aqueous silver nitrate.

Attempted Preparation of Heptaacetylstreptidine via the Dihydrochloride

A solution of dodecaacetyldihydrostreptomycin (25.4 g, 0.026 mole) in absolute chloroform (250 ml) was cooled in an ice bath and was saturated with dry hydrogen chloride. The solution was sealed in a flask and allowed to warm to room temperature. After 14 hr a gum had deposited on the surface of the solution. The chloroform was decanted and was discarded. The remaining gum was washed with fresh chloroform (2 x 20 ml), and it was then dried *in vacuo*. The product (11.4 g, 70%) was suspended in chloroform, and the suspension was shaken with a cold solution of saturated bicarbonate (2 x 30 ml); the sodium bicarbonate solution was discarded. The chloroform solution was dried over anhydrous

magnesium sulfate and was concentrated in vacuo at 50°. The product (6.52 g) showed three components by GC analysis of its TMS derivatives. (3% SE-30, argon inlet pressure 30 psig, C.T. 250°); R.T. 12.1, 14.2, 17.8 min.

Chromatography of the crude heptaacetylstreptidine over dried silicic acid (25 g, column 46 cm x 2.5 cm) using absolute chloroform failed to yield any material. Gradient elution with dry chloroform/dry ethyl acetate also failed to yield any material. The compound was finally eluted with dry dimethylformamide to yield an oil (5.95 g). GC analysis as above showed the presence of the same components as before. No further purification attempts were made.

Tri-O-acetylstreptidine Dihydrobromide

Dodecaacetyldihydrostreptomycin (45 g, 0.046 mole) was dissolved in chloroform (700 ml), and the solution was cooled in an ice bath. After treatment with 32% hydrogen bromide in acetic acid (30 ml), the solution was allowed to stand in the cold for 24 hr. The chloroform was decanted from the resulting oil, and the chloroform supernatent liquid was retreated with 32% hydrogen bromide in acetic acid (30 ml). After standing for 24 hr. in the cold, the chloroform was decanted from the resulting oil. The oils were combined, washed with fresh chloroform, and dried in vacuo. The dried material was dissolved in hot methanol (40 ml) and treated with activated carbon. The mixture was filtered, and the filtrate was heated with an excess of silver oxide for 30 minutes. The silver oxide was removed by filtration, and the methanol solution was slowly added to stirred ether (300 ml). The resulting precipitate was collected

and washed with fresh ether. Repeated treatment with carbon and precipitation from methanol as above gave a white, amorphous solid (8.5 g, 32%) which gave satisfactory analysis for tri-O-acetylstreptidine hydrobromide hydrate.

Anal.	$C_{14}H_{25}N_6O_7 \cdot HBR \cdot H_2O$ (548)	Calc'd:	C, 29.59; H, 4.97; N, 14.79; Br, 28.13
		Found:	C, 29.63; H, 5.09; N, 14.71; Br, 27.95

The infrared spectrum (pellet) showed absorptions at 3.0 and 5.85μ among others. The n.m.r. spectrum showed only three acetyl absorptions. The preparation of tri-O-acetylstreptidine hydrobromide hydrate was not found to be reproducible.

Dodecabenzoyldihydrostreptomycin

Dihydrostreptomycin sulfate (50 g, 0.041 mole) was benzoylated according to the procedure of Todd (35) to yield dodecabenzoyldihydrostreptomycin (115 g, 94%) as an amorphous solid, mp 135-137° [Lit. 137-141° (35)], $[\alpha]_D$ +79 ± 2 (c 2.0 chloroform) [Lit. [α] + 83° ± 2 (c 2.0 chloroform) (35)].

Heptabenzoylstreptidine

Dodecabenzoyldihydrostreptomycin (70 g, 0.0405 mole) was cleaved using 32% hydrogen bromide in acetic acid according to the procedure of Todd (35) to yield heptabenzoylstreptidine (20.1 g, 60%); mp 244-248° [Lit. 251-252° (35), $[\alpha]_D$ + 51° ± 2° (c 1.0 chloroform [Lit. + 53 ± 2° (c 1.0 chloroform) (35)].

Ethyl Thiostreptobiosaminide Diethyl Mercaptal Hydrochloride

Streptomycin hydrochloride (10 g, 0.014 mole) was degraded with

ethyl mercaptan (40 ml) according to the procedure of Kuehl et al. (14) to yield, after crystallization from water and recrystallization from ethyl acetate, ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride (6.2 g, 80%), mp 109-111° [Lit. 110-112° (14), $[\alpha]_D$ -258° ± 2.0° (c 1.50 methanol), [Lit. $[\alpha]_D$ -262° (14)]. The infrared spectrum (pellet) showed absorptions at 2.80, 3.35, 4.20, 5.87, and 6.10μ among others. The mass spectrum showed peaks at m/e (relative intensity) 487(0.01), 426(14), 352 (100) and 176(56), among others.

Ethyl N-Carbobenzoxythiostreptobiosaminide Diethyl Mercaptal

A solution of ethyl thiostreptobiosaminide diethyl mercaptal (1.0 g, 0.002 mole), water (20 ml), and methanol (5 ml) was adjusted to pH 8 with IR-400 (OH^-) resin (Amberlite). Carbobenzoxy chloride (7 ml, 80% by n.m.r. analysis, 0.032 mole) was added slowly with stirring. After an additional hour of stirring the resin was removed from the mixture by filtration, and the filtrate was extracted with ether (4 x 30 ml). The ether was dried over anhydrous magnesium sulfate, the magnesium sulfate was removed by filtration, and the filtrate was reduced to an oil in vacuo. The oil was triturated with petroleum ether (30-60°), and the resulting white, amorphous solid was crystallied from ethyl acetate to yield ethyl N-carbobenzoxythiostreptobiosaminide diethyl mercaptal: (0.6 g, 50%), mp 147-149°, $[\alpha]_D$ -200° ± 1.0° (c 1.0 methanol).

Anal. $C_{27}H_{43}O_9NS_3$ (621) Calc'd: C, 52.38; H, 6.78; N, 2.26; S, 15.49; Found: C, 52.40; H, 6.76; N, 2.32; S, 14.68

The infrared spectrum (pellet) showed absorptions at 2.80, 3.35, 4.20, 5.85, and 6.10 μ, among others. The n.m.r. spectrum (20%, deuterochloroform) showed readily identifiable absorptions at τ 8.6-9.0 (12H, complex) 7.32 (6H, quartet, J=7.4), 6.88 (3H, singlet) and 2.55 (5H, singlet). The mass spectrum shows peaks at m/e (relative intensity) 485(5),

344(76), 284(46), 231(100), and 91(80), among others.

Methyl α-Dihydrostreptobiosaminide Hydrochloride

Methyl α-dihydrostreptobiosaminide hydrochloride was prepared by the method of Fried and Wintersteiner (36). It was found that the resulting preparation could be further purified by chromatography over alumina.

Crude methyl α-dihydrostreptobiosaminide hydrochloride (25 g) was dissolved in methanol-benzene (50%, v/v), and the solution was placed on an aluminum column (800 g, 80 x 5 cm) prepared in the same solvent. Elution with methanol-benzene (50%, v/v) removed trace amounts of colored material. Elution with methanol yielded a colorless oil (15.12 g), which, upon crystallization from methanol-benzene, gave methyl α-dihydrostreptobiosaminide hydrochloride (10.6 g), mp 206-207° [Lit. 183-184° (36)]. $[\alpha]_D$ = -180° ± 2° (c 1.0 methanol) [Lit. $[\alpha]_D$ -135° (c 1.5 methanol) (36)].

Anal.	$C_{14}H_{27}O_9N \cdot HCl$ (373.9)	Calc'd:	C, 44.96; H, 7.59; N, 3.76; Cl, 9.47
	$C_{12}H_{27}O_9N \cdot H_2O$ (392)	Calc'd:	C, 42.88; H, 7.47; N, 3.58; Cl, 9.04
		Found:	C, 43.21; H, 7.35; N, 3.58; Cl, 9.05.

The infrared spectrum (pellet) showed absorptions at 2.75, 3.33, 4.12, and 5.83 μ, among others. The n.m.r. spectrum (20% in deuterium oxide) showed readily assignable absorptions at τ 8.95 (3H, doublet, J= 6.40), 7.13 (3H, singlet), 6.58 (3H, singlet), 4.94 (1H, doublet, J=3.25), and 4.41 (1H, doublet, J=3.41). Major peaks in the mass spectrum occurred at m/e (relative intensity) 353(4), 336(3), 322(80), and 276(100).

Methyl Pentaacetyl-α-dihydrostreptobiosaminide

Methyl α-dihydrostreptobiosaminide hydrochloride (1.0 g, 0.0026 mole) was acetylated using the procedure of Brink et al. (13). This procedure gave, after recrystallization from alcohol, methyl pentaacetyl-α-dihydrostreptobiosaminide (1.1 g, 67%), mp 197-200° [Lit. 198-198.5°, 193-194°, and 194-195° (13,15,36)]; $[\alpha]_D$ -126° ± 1.5° (c 1.0 chloroform), [Lit. $[\alpha]_D$ -117°, -120°, and -117° (13,15,36)]. The infrared spectrum (pellet) showed absorptions at 2.86, 3.34, 5.74, 5.85, and 6.18 μ, among others. The n.m.r. spectrum showed absorptions at τ 8.73 (3H, doublet, J=6.3), 7.97, 7.94, 7.86, 7.85, 7.28 (15H, all singlets), 6.95 (3H, singlet), 6.56 (3H, singlet), 5.7-6.0 (complex), and 4.2-5.2 (complex). Major peaks in the mass spectrum occurred at m/e (relative intensity) 532(5), 344(65), 284(53), 231(50), 98(100), and 43(76).

Ethyl Thiodihydrostreptobiosaminide Hydrochloride

A mixture of methyl α-dihydrostreptobiosaminide hydrochloride (1.0 g, 0.0026 mole) and ethyl mercaptan (10ml) was cooled in an ice bath. The mixture was then saturated with dry hydrogen chloride. The mixture was stirred overnight at room temperature, the excess ethyl mercaptan was distilled from the mixture, and the residue was dried in vacuo to yield ethyl thiodihydrostreptobiosaminide hydrochloride (1.08 g, 100%). Crystallization of the material from methanol-ethyl acetate gave a crystalline solid, mp 200-202° with charring at 195°.

Anal.	$C_{15}H_{30}NO_8SCl$ (419.9)	Calc'd:	C, 42.90; H, 7.12; N, 3.24; S, 7.65; Cl, 8.44
		Found:	C, 43.05; H, 7.18; N, 3.24; S, 7.81; Cl, 8.22

The infrared spectrum (pellet) showed absorptions at 2.83, 3.33, 4.12, 5.84, and 6.39 μ, among others. The n.m.r. spectrum (20% dimethylsulfoxide-d_6) showed absorptions at τ 8.96 (3H, doublet, J=6.4), 8.91 (3H, triplet, J=7.2), 7.28 (2H, quartet, J=7.2), 7.14 (3H, singlet), 5.6-6.9 (complex), 4.70 (1H, doublet, J=5.26), and 4.44 (1H, doublet, J= 3.41). Major peaks in the mass spectrum occurred at m/e (relative intensity) 383(<0.1), 354(10), 352(10), 322(100), 304(10), and 176(85).

Ethyl N-Carbobenzoxythiodihydrostreptobiosaminide

A stirred solution of ethyl thiodihydrostreptobiosaminide hydrochloride (2.0 g, 0.0048 mole) in water (15 ml) was treated with an excess of sodium bicarbonate, and carbobenzoxy chloride (10 ml, 90% by n.m.r. analysis, 0.051 mole) was slowly added to the solution over a period of 20 min. An additional two hours of stirring resulted in the formation of a gummy solid. The solvents were removed in vacuo at room temperature. The resulting solid was triturated with ether (2 x 20 ml). The ether was discarded. The solid was then extracted with hot methanol (4 x 20 ml). The methanol extractions were pooled and reduced to dryness in vacuo at 50°. The resulting solid was treated with hot ethyl acetate (25 ml), and the mixture was filtered while hot. Upon cooling, the filtrate yielded crystals of ethyl N-carbobenzoxythiodihydrostreptobiosaminide (1.3 g, 52%) mp 169-170°, $[\alpha]_D$ -178° ± 2° (c 1.0, methanol).

Anal. $C_{23}H_{35}NO_{10}S$ (517.65) Calc'd: C, 54.48; H, 6.82; N, 2.71; S, 6.20
Found: C, 54.22; H, 6.73; N, 3.02; S, 7.32

The infrared spectrum (pellet) showed absorptions at 2.80, 3.35, 4.26, and 5.97 μ, among others. The n.m.r. spectrum (20% dimethylsulfoxide-d_6)

showed readily assignable absorptions at τ 8.94 (3H, doublet, J=6.5), 8.81 (3H, triplet, J=7.1), 7.40 (2H, quartet, J=7.1), 6.84 (3H, singlet), and 2.54 (5H, singlet). Major peaks in the mass spectrum occurred at m/e (relative intensity) 456(18), 310(19), 108(42), 107(34), 91(67), 79(34), 77(27), 45(35), 43(100), and 29(52), among others.

Ethyl Tetra-O-acetyl-N-carbobenzoxythiodihydrostreptobiosaminide

Acetic anhydride (100 ml, 1.07 moles) was slowly added to a solution of ethyl N-carbobenzoxythiodihydrostreptobiosaminide (10 g, 0.019 mole) and pyridine (100 ml), and the solution was allowed to stand at room temperature for 44 hr. After this period, the solvents were removed in vacuo, and the residue was dissolved in chloroform (50 ml). The chloroform solution was washed with a saturated solution of sodium bicarbonate (3 x 20 ml) followed by distilled water (20 ml), and was then dried over magnesium sulfate. The magnesium sulfate was removed by filtration, and the chloroform solution was concentrated in vacuo at 50°. The resulting oil (14 g, 106%) was chromatographed over silicic acid (200 g, column 46 cn x 4.7 cm) using chloroform. The product (12.2 g, 92%) was eluted in fractions (fraction volume, 20 ml) 3-10. The product resisted all crystallization attempts. The n.m.r. spectrum (≈20%, carbon tetrachloride) showed readily assignable absorptions at τ 8.77 (3H, doublet, J= 6.5), 8.71 (3H, triplet, J=7.1), 8.12, 8.07, 8.04, 7.97 (12H, all singlets), 7.40 (2H, quartet, J=7.1), 7.06 (3H, singlet), and 2.67 (5H, singlet). No further attempts were made to purify this compound.

Ethyl Tetra-O-benzoyl-N-carbobenzoxylthiodihydrostreptobiosaminide

Benzoyl chloride (3.5 ml, 0.03 mole) was slowly added to a solu-

tion of ethyl N-carbobenzoxythiodihydrostreptobiosaminide (3.0 g, 0.006 mole) and pyridine (20 ml) maintained at 0° by external cooling. The mixture was then stirred at room temperature for 14 hr. Water (1.0 ml) was added to the mixture, and the resulting residue was stirred for an additional hour. The solution was then diluted with chloroform (30 ml), and the solution was extracted with a saturated solution of sodium bicarbonate until no release of carbon dioxide was detected. The solution was finally washed with distilled water (20 ml) and dried over anhydrous magnesium sulfate. The magnesium sulfate was removed by filtration, and the filtrate was evaporated *in vacuo* at 60°. The resulting oil (6.0 g, 111%) was chromatographed over silicic acid (20 g, column 26.5 cm x 2.3 cm) using methanol-chloroform (5% v/v). The product (4.3 g, 80%) was eluted in fractions (fraction volume, 20 ml) 3-7. Crystallization of the compound from alcohol gave a solid melting at 133-135° $[\alpha]_D$ -33° ± 2° (*c* 1.0 chloroform).

Anal. $C_{51}H_{51}NO_{14}S$	Calc'd:	C, 65.58; H, 5.50; N, 1.50; S, 3.43
(934)	Found:	C, 65.62; H, 5.72; N, 0.92; S, 2.32 C, 65.56, H, 5.55

The infrared spectrum (pellet) showed absorptions at 2.91, 3.40, 5.82 and 6.25 μ, among others. The n.m.r. spectrum (20% in carbon tetrachloride) showed readily assignable absorptions at τ 8.75 (3H, doublet, *J*=6.5), 8.80 (3H, triplet, *J*=7.3), 7.50 (2H, quartet, *J*=7.3), 7.15 (1H, singlet: this absorption shifted to higher field on dilution) 6.93 (3H, singlet), 4.08 (2H, singlet), 2.99 (5H, singlet), 2.98-2.50 (12H, complex multiplet), and 2.20-1.88 (8H, complex multiplet).

Methyl Tetra-O-benzoyl-N-carbobenzoxydihydrostreptobiosaminide

A solution of bromine (0.08 ml, 0.0015 mole) in anhydrous carbon tetrachloride (10 ml) was slowly added to a stirred mixture of ethyl tetra-O-benzoyl-N-carbobenzoxythiodihydrostreptobiosaminide (1.0 g, 0.00107 mole), anhydrous carbon tetrachloride (20 ml), and silver carbonate (1.4 g) in the presence of anhydrous magnesium sulfate. After the mixture was stirred at room temperature for 16 hr, the mixture was filtered, and the filtrate was concentrated in vacuo at 50°. The residue (0.97 g, 100%) was chromatographed over silicic acid (20 g, column 26.5 cm x 2.3 cm) using chloroform. The product was eluted in fractions (fraction volume, 20 ml) 3-8. Crystallization of the pooled fractions from alcohol gave methyl tetra-O-benzoyl-N-carbobenzoxydihydrostreptobiosaminide (0.64 g, 67%), mp 166-167°, $[\alpha]_D$ 24° ± 2° (c 1.0 chloroform).

Anal. $C_{50}H_{49}NO_{15}$ (903.9) Calc'd: C, 66.44; H, 5.46; N, 1.55
Found: C, 66.55; N, 5.46; N, 1.70

The infrared spectrum (pellet) showed absorptions at 2.92, 3.41, 5.81, 5.92, and 6.25 μ, among others. The n.m.r. spectrum (20% in deuteriochloroform) showed readily assignable absorptions τ 8.65 (3H, doublet, J=6.6), 6.84 (3H, singlet), 6.56 (3H, singlet), 6.50 (1H, singlet, shifts to higher field on dilution), 2.82 (5H, singlet) 2.75-2.42 (12H, complex multiplet), and 2.20-1.99 (8H, complex multiplet).

Cyclohexyl Tetra-O-benzoyl-N-carbobenzoxydihydrostreptobiosaminide

A solution of bromine (0.08 ml, 0.0015 mole) in anhydrous carbon tetrachloride (10 ml) was slowly added to a stirred mixture of ethyl tetra-O-benzoyl-N-carbobenzoxythiodihydrostreptobiosaminide (1.0 g,

0.00107 mole), anhydrous cyclohexanol (0.54 ml, 0.0054 mole), anhydrous carbon tetrachloride (20 ml) and silver carbonate (1.4 g) in the presence of anhydrous magnesium sulfate. After the mixture was stirred at room temperature for 16 hr it was filtered, and the filtrate was evaporated *in vacuo* at 50°. The residue (1.1 g, 104%) was chromatographed over silicic acid (20 g, column 26.5 cm x 2.3 cm) using chloroform. The product was eluted in fractions (fraction volume 20 ml) 2-7. Crystallization of the pooled fractions from alcohol gave cyclohexyl tetra-*O*-benzoyl-*N*-carbobenzoxydihydrostreptobiosaminide (0.72 g, 70%); mp 165-167°, $[\alpha]_D$ 20° ± 2° (*c* 1.0 chloroform).

Anal. $C_{55}H_{57}NO_{15}$ (972.1) Calc'd: C, 67.96; H, 5.91; N, 1.44
Found: C, 67.92; H, 6.52; N, 1.53

The infrared spectrum (pellet) showed absorptions at 2.91, 3.42, 5.84, 5.89, and 6.25 μ, among others. The n.m.r. spectrum (20% in carbon tetrachloride) showed readily assignable absorptions at τ 8.92-8.00, 8.70 (14H total, broad multiplet, doublet, *J*=6.3), 6.87 (3H, singlet), 6.40 (1H, singlet), 2.99 (5H, singlet), 2.83-2.65 (12H, complex multiplet), and 2.25-1.89 (8H, complex multiplet).

Attempted Reaction of Heptabenzoylstreptidine with Ethyl Tetra-*O*-benzoyl-*N*-carbobenzoxythiodihydrostreptobiosaminide

A solution of bromine (0.04 ml, 0.00075 mole) in anhydrous chloroform (10 ml) was slowly added to a stirred mixture of ethyl tetra-*O*-benzoyl-*N*-carbobenzoxythiodihydrostreptobiosaminide (0.50 g, 0.00053 mole), hetabenzoylstreptidine (0.525 g, 0.00053 mole), anhydrous chloroform (20 ml), and silver carbonate (0.7 g) in the presence of anhydrous mag-

nesium sulfate. After the mixture was stirred at room temperature for 16 hr, it was filtered, and the filtrate was evaporated *in vacuo* at 50°. The residue (1.36 g, 110%) was chromatographed over silicic acid (20 g, column 26.5 cm x 2.3 cm) using chloroform. The product (0.51 g) was eluted in fractions 2-6 (fraction volume, 20 ml). After crystallization of the pooled fractions from methanol/benzene the material showed spectral properties identical to that of heptabenzoylstreptidine; mp 248-250°; [Lit. (35) 250-252°].

Attempted Reaction of Ethyl Tetra-*O*-benzoyl-*N*-carbobenzoxy-α-thiodihydrostreptobiosaminide with Heptabenzoylstreptidine in *N*,*N*-Dimethylformamide

A solution of bromine (0.04 ml, 0.00075 mole) in anhydrous dimethylformamide (15 ml) was slowly added to a mixture of tetra-*O*-benzoyl-*N*-carbobenzoxy-α-thiodihydrostreptobiosaminide (0.5 g, 0.00053 mole), anhydrous dimethylformamide (20 ml), heptabenzoylstreptidine (0.53 g, 0.00075 mole), and silver carbonate (0.8 g) in the presence of Drierite. The reaction mixture was sealed from the light and stirred for 16 hr. The mixture was filtered, and the filtrate was concentrated *in vacuo* at 70°. The residue was extracted with chloroform (3 x 15 ml), and the chloroform solution was concentrated and chromatographed over silicic acid (20 g, column dimensions: 26.5 cm x 2.3 cm) using chloroform. The product (0.51 g) was eluted in fractions (fraction volume: 20 ml) 3-7. The eluted material was crystallized (0.39 g) from methanol/benzene and showed physical and spectral properties identical with heptabenzoylstreptidine.

Attempted Reaction of Tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide with Heptabenzoylstreptidine in 1,2-Dimethoxyethane

A solution of bromine (0.08 ml, 0.0015 mole) in anhydrous dimethoxyethane (15 ml) was slowly added to a mixture of tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide (1.0 g, 0.00107 mole), anhydrous dimethoxyethane (30 ml), heptabenzoylstreptidine (1.06 g, 0.00107 mole), and silver carbonate (1.4 g) in the presence of anhydrous magnesium sulfate. After stirring for 16 hr at room temperature in the absence of light, mixture was filtered, and the filtrate was concentrated in vacuo at 50°. The residue (2.2 g, 107%) was chromatographed over silicic acid (20 g, column dimensions: 26.5 cm x 2.3 cm) using chloroform. The product (1.1 g) was eluted in fractions (fraction volume: 20 ml) 2-8. The n.m.r. spectrum of the material (20% in deuteriochloroform) showed absorption characteristic of both dihydrostreptobiosamine and streptidine. Removal of the benzoyl groups with methanol/ammonia followed by reduction as previously described gave a material which showed only one Weber positive spot; this corresponded to streptidine by paper chromatography.

Attempted Reaction of Tetra-O-benzoyl-N-carbobenzoyl-α-thiodihydrostreptobiosaminide with Heptaacetylstreptidine

A solution of bromine (0.04 ml, 0.00075 mole) in anhydrous dimethylformamide (15 ml) was slowly added to a stirred mixture of tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide, crude hepaacetylstreptidine dihydrobromide (10 g), anhydrous dimethylformamide (20 ml), and silver carbonate (1.4 g) in the presence of Drierite. The reaction mixture was filtered, and the filtrate was concentrated in vacuo at 70°. The

residue was extracted with chloroform (5 x 20 ml) and the chloroform solution was concentrated to an oil. The oil was chromatographed over silicic acid (20 g, column dimensions: 26.5 cm x 2.3 cm) using chloroform. No material was obtained from the first twelve fractions (fraction volume: 20 ml). Elution with 5% methanol/chloroform (v/v) gave an oil (0.46 g) which showed benzoyl- but little acetyl- absorption in the n.m.r. spectrum. The protecting groups were removed from the material as previously described to yield an amorphous solid that gave a negative Weber reaction. No further attempt was made to identify this material.

CHAPTER III

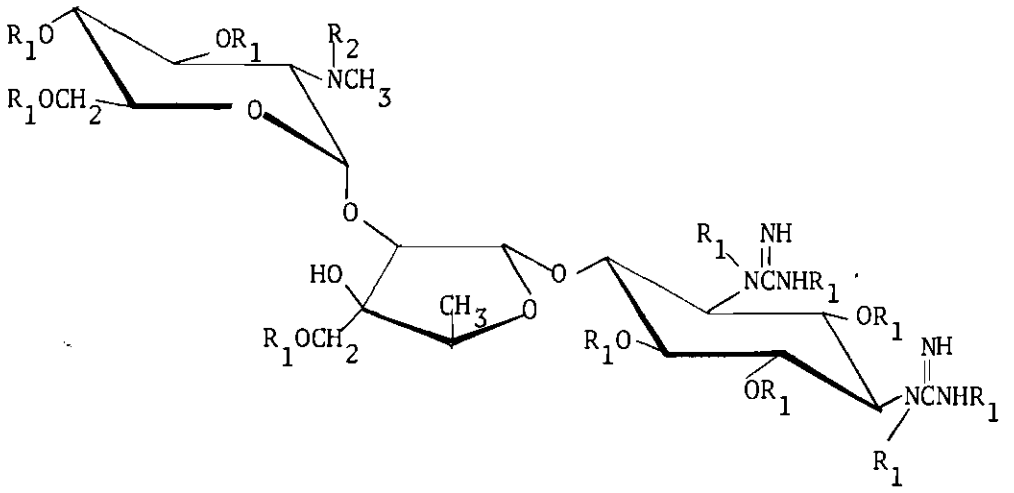
DISCUSSION OF RESULTS

The purpose of this research was to achieve a glycosidic union between the moieties of dihydrostreptomycin: streptidine and dihydrostreptobiosamine. Conditions were to be sought that would allow the preparation of the more difficulty obtainable α-configuration between these two fragments.

The linkage of these two fragments must involve the preparation of moieties whose interfering functional groups are inactivated by a blocking group. Likewise, it is necessary that these blocking groups be of such a nature that they can be removed once the desired reaction has taken place. Therefore, before any investigation involving the glycosidic synthesis of the two dihydrostreptomycin derivatives was undertaken, a study of suitable protecting groups was initiated.

The classical protecting group used in carbohydrate chemistry is the acetyl group. Acetyl derivatives of sugars are easily prepared, generally crystalline, and the acetyl groups are readily removed by treatment with mild base. Dihydrostreptomycin, I, contains three types of acetylatable functional groups: hydroxyl, guanidino, and amino. As noted above, ester acetyl groups are readily hydrolyzed by mild base. Acetylated guanidines may be considered as a nitrogen analog of an anhydride, and thus, they would be expected to be hydrolyzed rapidly by base. Amides, however, are generally resistant to hydrolysis and require the use

of strong acid or base to regenerate the free amine. It is not surprising, then, that Stenlake (34) reports treatment of peracetyldihydrostreptomycin, IX, with methanol saturated with ammonia yields N-acetyldi-



IX $R_1 = R_2 = \overset{O}{\overset{\|}{C}}CH_3$

X $R_1 = H_1$, $R_2 = \overset{O}{\overset{\|}{C}}CH_3$

XI $R_1 = H$, $R_2 = \overset{O}{\overset{\|}{C}}OCH_2C_6H_5$

XII $R_1 = \overset{O}{\overset{\|}{C}}C_6H_5$, $R_2 = \overset{O}{\overset{\|}{C}}OCH_2C_6H_5$

XIII $R_1 = R_2 = \overset{O}{\overset{\|}{C}}C_6H_5$

hydrostreptomycin, X. Because of the ease of hydrolysis of the guanidino groups of dihydrostreptomycin, the choice of base that one may use to hydrolyze an amide is limited. A stronger base than methanol saturated with ammonia and yet nondestructive to the guanidino group is liquid ammonia at room temperature for several days. All the O-acetyl groups were removed but no significant hydrolysis of the N-acetyl group of the N-methyl-L-glucosamine fragment, could be detected by n.m.r. analysis of the product (37).

The inability to remove under mild conditions the N-acetyl group from the N-methyl-L-glucosamine portion of peracetyldihydrostreptomycin necessitated the search for a different means of masking the amino function. In amino acid chemistry the carbobenzoxy group has been found very effective as a blocking group for the amino function. It has been shown to react selectively with amines in the presence of hydroxyl groups and can readily be removed by hydrogenolysis. In dihydrostreptomycin the two guanidino groups would not be expected to participate in the reaction because of the much greater basicity of these groups as compared to that of the N-methylamino group. Examples of the selectivity of carbobenzoxylation can be found in the chemistry of serine and arginine. The N-carbobenzoxy derivative of both of these compounds can be formed readily in the presence of a mild base such as sodium bicarbonate. However, O,N-dicarbobenzoxyserine can be prepared only with difficulty, and the carbobenzoxylation of the guanidino group of arginine requires the use of 4 N base (38).

In a recent study of the relationship of the cationic groups of dihydrostreptomycin to its biological activity, Polglase (39) prepared N-

carbobenzoxydihydrostreptomycin, XI, and he was able to remove this protecting group without any loss in biological potency. This demonstration removed all difficulties associated with the blocking of the N-methylamino function in dihydrostreptomycin.

The possibility that benzoyl derivatives of the moieties, dihydrostreptobiosamine and streptidine, would be found more convenient in the glycosidic syntheses gave rise to a study of the removal of this protecting group from dihydrostreptomycin. Whereas Polglase (39) had treated dihydrostreptomycin with carbobenzoxy chloride in the presence of magnesium oxide, this method was found unsatisfactory because of the difficulty of separating small amounts of water-soluble magnesium salts from the product. Thus the N-carbobenzoxy derivative of dihydrostreptomycin was prepared by a modification of this procedure.

The Amberlite anion exchange resin, IR-400 (OH^-), was found to be a more convenient base to use in the reaction since it could be removed quantitatively at the completion of the reaction. The reaction was found to be dependent on pH and only at pH 10 could 100% carbobenzoxylation (based on n.m.r. analysis) be achieved. The n.m.r. spectrum of the product was consistent with the expected structure. As expected, the absorption of the N-methyl group of the carbobenzoxylated N-methylamino group was shifted downfield 0.15 ppm (6.95 vs 7.10) relative to the N-methyl group of dihydrostreptomycin itself. The agreement of the integration of the C-methyl and N-methyl groups to that of the phenyl protons plus the appearance of a single N-methyl absorption confirmed the quantitative carbobenzoxylation of dihydrostreptomycin.

Carbobenzoxylated dihydrostreptomycin was benzoylated according to the procedure of Todd (35). Since the purpose of this study was to determine a method of removing the O-benzoyl groups, no attempt was made to remove partially benzoylated material from the preparation of O-benzoylated N-carbobenzoxydihydrostreptomycin, XII.

Because methanol saturated with ammonia had been successful in the hydrolysis of O-acetyl groups from dihydrostreptomycin (34), the same procedure was repeated with N-carbobenzoxy poly-O-benzoyldihydrostreptomycin, XII. Treatment of this compound for three days at room temperature with methanol saturated with ammonia clearly removed the benzoyl groups. The methyl benzoate and benzamide could easily be removed by washing the crude N-carbobenzoxydihydrostreptomycin, XI, with ether. The presence of two N-methyl signals in the n.m.r. spectrum indicated that the carbobenzoxy group had also been partially hydrolyzed. Since great difficulty was encounted by Chawla (40) in attempts to separate the carbobenzoxy derivative of α-dihydrostreptomycin from β-dihydrostreptomycin, and since complete decarbobenzoxylation of XI would result in homogeneous material, no purification was attempted at this stage.

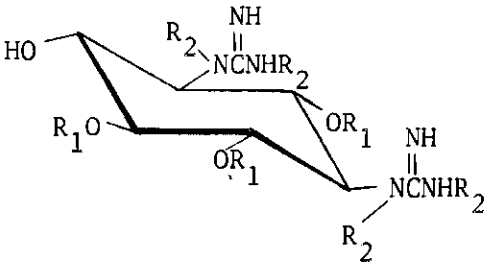
The carbobenzoxy group is generally removed by hydrogenolysis at atmospheric pressure; however, in the case of N-carbobenzoxydihydrostreptomycin better results were obtained at an initial hydrogen pressure of approximately two pounds per square inch. The recovery of dihydrostreptomycin was dependent on the solvent used. Since dihydrostreptomycin is partially absorbed on carbon when 50% methanol/water (v/v) is used as the solvent, a poor recovery of material was obtained. The yield was increased significantly when the solvent was changed to anhydrous methanol.

After forming the sulfate salt by passing the recovered dihydro-

streptomycin over a column of Amberlite anion exchange resin IR-45 ($SO_4^{=}$), a papergram of the material was run to determine the homogenity of the material. Development of the papergram according to Hedig (31) showed two Weber (41) positive spots, R_F 0.78 and 0.00 (authentic dihydrostreptomycin sulfate shows an R_F of 0.78 in this system). No attempt was made to identify the R_F 0.00 material. Separation of these two components was achieved by placing them on a column of Sephadex G-15 and eluting with 0.01 N formic acid. The eluted material (R_F 0.78) showed an n.m.r. spectrum identical to that of authentic dihydrostreptomycin sulfate. As a further comparison of identity, the trihelianthate salt of the recovered dihydrostreptomycin sulfate was prepared and found to have an identical IR spectrum to that of an authentic sample.

With the determination that either O-acetyl or O-benzoyl groups were satisfactory as blocking groups for the glycosidic synthesis of dihydrostreptomycin, attention was turned to the preparation of the reported compounds, heptaacetyl- and heptabenzoylstreptidine.

Heptabenzoylstreptidine, XIV, is a crystalline compound that can easily be prepared from the acid hydrolysis of crude polybenzoyldihydrostreptomycin, XIII. This preparation has been carried out many times in



XIV $R_1 = R_2 = \overset{O}{\overset{\|}{C}}C_6H_5$

XV $R_1 = R_2 = \overset{O}{\overset{\|}{C}}CH_3$

XVI $R_1 = \overset{O}{\overset{\|}{C}}CH_3$, $R_2 = H$

this laboratory and a discussion of the results can be found elsewhere (35). On the other hand, the preparation of heptaacetylstreptidine, XV, was not found possible.

It seems reasonable that if acid hydrolysis of polybenzoyldihydrostreptomycin results in the cleavage of heptabenzoylstreptidine, then, the same procedure should yield heptaaceylstreptidine from polyacetyldihydrostreptomycin. Indeed, Stenlake reported (35) that treatment of polyacetyldihydrostreptomycin with hydrogen bromide in anhydrous acetic acid resulted in the formation of micro-crystalline heptaacetylstreptidine dihydrobromide, XV. Unfortunately, these results could not be confirmed during the course of this research.

Although polyacetyldihydrostreptomycin (IX) has been prepared from dihydrostreptomycin (11) using a two stage acetylation procedure with acetic anhydride/pyridine, attempts to apply these methods to dihydrostreptomycin sulfate gave very poor yields of crude material. The compound was finally prepared by the method of Stenlake (34) in which anhydrous sodium acetate was added to assist in the removal of the sulfate ion. According to Stenlake, repeated precipitation of the material by the slow addition of a benzene solution to petroleum ether (b.p. 80-100°) gave a higher yield of purified material than the previously reported chromatographic method (11). Attempts to purify polyacetyldihydrostreptomycin by repeated precipitation resulted in material that had the same optical rotation ($[\alpha]_D$ -64° ± 2 [Lit: $[\alpha]_D$ -64.5 (11), $[\alpha]_D$ -67° (34)]) as that reported for "pure" polyacetyldihydrostreptomycin, but the material showed at least four components by T.L.C. However, since no difficulty was encountered in the preparation of heptabenzoylstreptidine from

crude polybenzoyldihydrostreptomycin (35) no further purification of the polyacetyl dihydrostreptomycin seemed necessary.

Cleavage of polyacetyldihydrostreptomycin with hydrogen bromide in acetic acid resulted in the formation of a crude oil. After removal of trace amounts of acid, the oil was dissolved in methanol and decolorlized with carbon; however, addition of the methanol solution to ether failed to yield colorless, micro-crystalline material as reported. Many repeated attempts to purify the material led to the isolation of a product that gave satisfactory analysis for tri-O-acetylstreptidine dihydrobromide, XVI. The infrared and n.m.r. spectra confirmed the presence of only O-acetyl groups. The procedure, however, was not found to be reproducible. Attempts to prepare heptaacetylstreptidine from the crude heptaacetylstreptidine dihydrobromide failed to yield any material that gave a negative silver nitrate test.

The failure to obtain any pure heptaacetylstreptidine dihydrobromide plus the isolation of tri-O-acetylstreptidine dihydrobromide indicated that the guanidino acetyl groups were much more labile to hydrolysis than expected. Since polyacetyldihydrostreptomycin was shown by T.L.C. to be a mixture of at least four components, the possibility existed that partial hydrolysis of the guanidino acetyl groups had occurred during workup. The preparation of polyacetyldihydrostreptomycin was repeated using dry and aprotic solvents at every step; however, no increase in purity was noted.

In an attempt to prepare heptaacetylstreptidine (XV) by a milder method, polyacetyldihydrostreptomycin was dissolved in absolute chloroform, and dry hydrogen chloride was passed into the solution. The re-

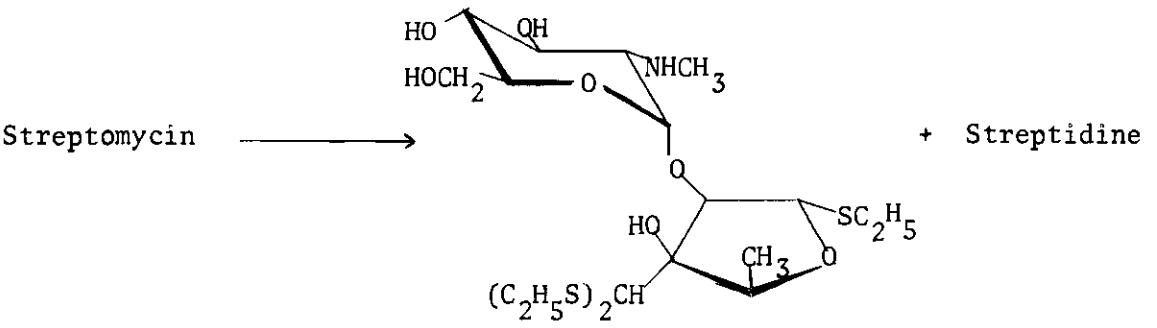
sulting crude heptaacetylstreptidine hydrochloride was suspended in chloroform, and the chloroform was extracted with cold saturated sodium bicarbonate solution. The chloroform was dried as quickly as possible over magnesium sulfate. The T.M.S. derivative of the resulting crude heptaacetylstreptidine was prepared and analyzed by gas/liquid chromatography. The preparation showed three components in the approximate ratio of 1:2:1. Attempted purification of the material by chromatography over silicic acid failed. The material eluted from the column showed the same components as the crude material when analysis was made of the T.M.S. derivative. No further attempts were made to prepare heptaacetylstreptidine.

The next step in the synthesis was the preparation of dihydrostreptobiosamine moiety. Any derivative of dihydrostreptobiosamine that is prepared must have the following features. First, an easily displaceable group must be attached at C-1 of the dihydrostreptose fragment. Second, the amino group of the N-methyl-L-flucosamine fragment must be blocked with the carbobenzoxy group. Third, the hydroxyl groups must be blocked by some easily removable group such as acetyl or benzoyl.

Classically, glycosyl halides, especially bromides, are used in glycosidic syntheses. However, very little work has been reported on the behavior of glycosyl halides of furanosides such as dihydrostreptose except that they are generally much less stable than the corresponding pyranosides (42). Also, few examples with accompanying experimental detail are known where glycosyl halides have been used to synthesize α-glycosides. Thus, it appeared that the direct preparation of a glycosyl bromide of dihydrostreptobiosamine was not the method of choice.

During the original study of the chemistry of streptomycin and dihydrostreptomycin, recourse was made of the known cleavage of glycosidic

bonds with acidic ethyl mercaptan. When streptomycin was treated with ethyl mercaptan saturated with dry hydrogen chloride, a crystalline compound, ethyl thiostreptobiosaminide diethylmercaptal hydrochloride (XVII) was isolated (14). The cleavage of only one of the glycosidic bonds of



XVII

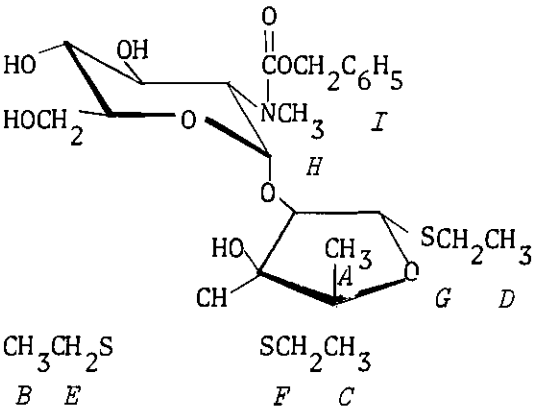
streptomycin is due to the presence of the protonated amine in the N-methyl-L-glucosamine fragment. The presence of a positive charge adjacent to C_1 hinders the approach of a proton to the N-methyl-L-glucosamine glycosyl bond. The ethylthio group, along with other thio groups, is an excellent precursor of glycosyl halides, and more recently (30), it has been shown that they can be displaced directly to form both α- and β-glycosides depending on the configuration of the original thio group.

Since it appeared that an ethyl thiodihydrostreptobiosamide derivative would be an acceptable moiety for the glycosidic synthesis of dihydrostreptomycin, the next step was to determine conditions for the preparation of the N-carbobenzoxy derivative. Since the mercaptolysis of dihydrostreptomycin gave an amorphous product, whereas the product from streptomycin was reported as a crystalline material, the latter was pre-

pared to use as a model for the carbobenzoxylation reaction.

Treatment of streptomycin hydrochloride with ethyl mercaptan saturated with dry hydrogen chloride gave XVII in good yield. Repeated recrystallization from water did not give material of satisfactory purity. Much better results were obtained when the compound was recrystallized from ethyl acetate. The physical properties of the compound agreed satisfactorily with those of the literature. The mass spectrum of compound XVII showed peaks at m/e (relative intensity) 487[0.1, M^+], 421[14, M - SC_2H_5], 352[100, M - $CH(SC_2H_5)$], and 176[56, M - dihydrostreptose fragment].

Carbobenzoxylation of ethyl thiostreptobiosaminide diethylmercaptal hydrochloride (XVII) was accomplished in a similar manner to that of dihydrostreptomycin. Amberlite anion exchange resin [IR-400(OH^-)] was used as the base. Because of the low solubility of the thio acetal in water, sufficient methanol was added to effect solution. Crystallization of the product, XVIII, from ethyl acetate gave material that showed satisfactory elemental analysis. The infrared and n.m.r. spectra were consistent with the expected structure. Absorptions in the n.m.r. spectrum that can be assigned are shown below.



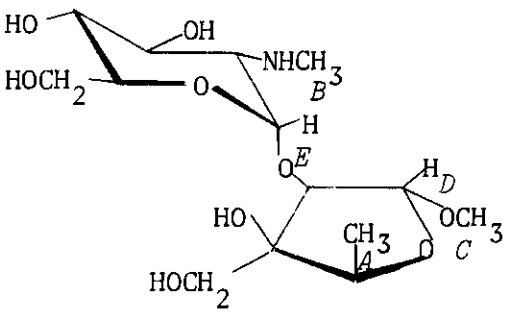
H	τ	Jcps
A,B,C,D,	8.6-9.0	
E,F,G	7.32	7.4
H	6.88	
I	2.55	

The mass spectrum of XVIII showed an assignable peak at m/e (relative intensity) 486[5, M - $CH(SC_2H_5)_2$], and unassignable peaks at m/e (relative intensity) 344[76], 284[46], and 231[100].

The mercaptolysis of dihydrostreptomycin is reported to yield an amorphous solid (43). When this procedure was repeated, crystalline material was obtained by various chromatographic techniques, but the yields varied from 0-70%. Because of unexplainable nonreproducibility of the reaction, the mercaptolysis of dihydrostreptomycin was abandoned in favor of preparing this compound from the known methyl α-dihydrostreptobiosaminide hydrochloride.

The methanolysis of dihydrostreptomycin hydrochloride (36) yields methyl α-dihydrostreptobiosaminide hydrochloride, XIX, as an amorphous solid. It was found that the material could be obtained crystalline by chromatography over alumina. However, in subsequent reactions, no difference in yield could be noted between the use of amorphous or crystalline material.

The crystalline methyl α-dihydrostreptobiosaminide hydrochloride (XIX) gave satisfactory elemental analysis. The n.m.r. absorptions which can be assigned are shown below.



XIX

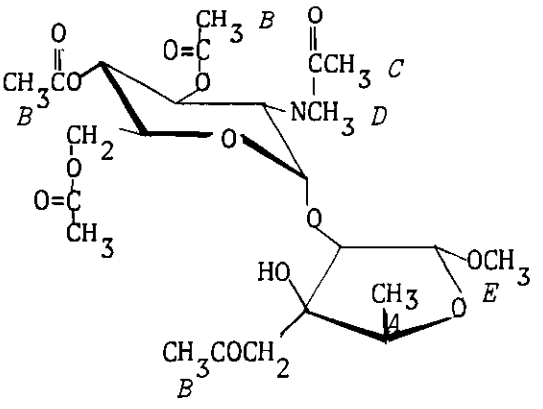
H	τ	Jcps
A	8.95	
B	7.13	
C	6.58	
D	4.94	3.25
E	4.41	3.41

The coupling constant of H_E is in agreement with that found in dihydrostreptomycin. The coupling constant of H_D indicates a trans relationship between the protons at C_1 and C_2 of the dihydrostreptose fragment, and thus confirms the assignment of the α-configuration for this compound.

The mass spectrum of XIX showed peaks at m/e (relative intensity) 353[4, M^+], 336[3, M - OH], 322[80, M - OCH_3]. The base peak at 276 could not be assigned.

Methyl α-dihydrostreptobiosaminide (XIX) hydrochloride was acetylated to give the known methyl pentaacetyl α-dihydrostreptobiosaminide (XX). All physical properties were in agreement with those reported in the literature. The infrared and n.m.r. spectra agreed with the structure.

Absorptions in the n.m.r. spectrum that can be assigned as shown below.

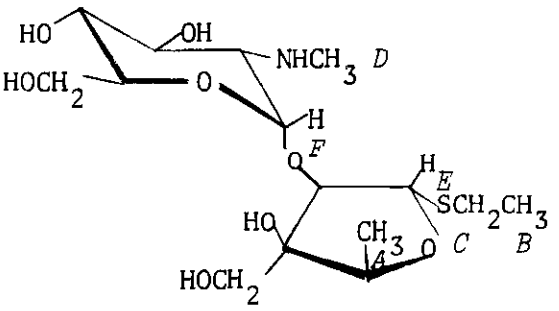


XX

H	τ	Jcps
A	8.73	6.3
B's	7.97	
	7.94	
	7.86	
	7.85	
C	7.28	
D	6.95	
E	6.56	

The mass spectrum of XX showed assignable peaks at m/e (relative intensity) at 532[5, M - OCH_3] and 344[65, M - dihydrostreptose fragment]. Other major peaks occurred at m/e (relative intensity) 284[53], 231[50] and 98[100].

Treatment of methyl α-dihydrostreptobiosaminide hydrochloride (XIX) with ethyl mercaptan saturated with dry hydrogen chloride gave ethyl thio-dihydrostreptobiosaminide hydrochloride, XXI, in quantitative yield. The material could be easily crystallized from ethyl acetate/methanol with little loss in yield. Satisfactory elemental analysis was obtained for the crystalline material. All spectral data were in agreement with the proposed structure. Absorption in the n.m.r. spectrum that can be assigned are shown below.



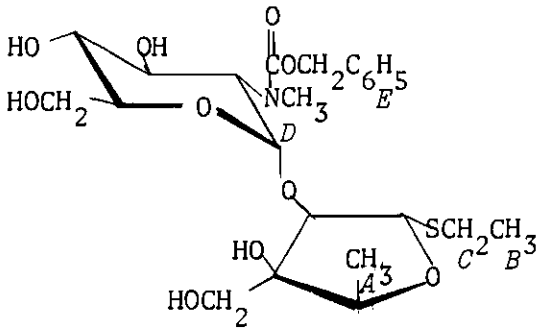
XXI

H	τ	Jcps
A	8.96	6.4
B	8.91	7.2
C	7.28	7.2
D	7.14	
E	4.70	5.26
F	4.44	3.41

The coupling constant of H_F is in agreement with that of dihydrostreptomycin. Unfortunately, the relationship between dihedral angles and coupling constants are not as well defined for five membered rings as for six membered rings. The magnitude of the coupling of H_E doesn't allow one to make an unequivocal assignment regarding the configuration about C_1 of the dihydrostreptose fragment. However, when the nature of the reaction is considered, i.e., under equilibrium conditions where the more stable product is formed, together with the fact that the same reaction with methanol, under the same conditions, yields the α-glycoside, it seems reasonable to assume that this compound also has the α-configuration. The unusually large coupling constant may be attributed to puckering of the ring by interaction between the large sulfur atom and other near by substituents.

The mass spectrum of XXI showed peaks at m/e (relative intensity) 383[0.1, M^+], 354[10, M - NCH_3], 352[10, M - CH_2OH], 322[100, M - SC_2H_5], 304[10, M - SC_2H_5 and H_2O] and 176[85, M - dihydrostreptose fragment].

Attempts to prepare the carbobenzoxy derivative of ethyl α-thiodihydrostreptobiosaminide hydrochloride (XXI) using Amberlite anion exchange resin [IR-400(OH^-)] failed because the desired product precipitated from the reaction mixture onto the surface of the resin. The use of sodium bicarbonate as the base, followed by a series of extractions of the resulting solids with hot methanol and hot ethyl acetate gave crystalline ethyl N-carbobenzoxy-α-thiodihydrostreptobiosaminide, XXII. Satisfactory elemental analysis was obtained for the compound, and all spectral properties were in agreement with the expected structure. Absorptions in the n.m.r. spectrum which may be assigned are shown below.

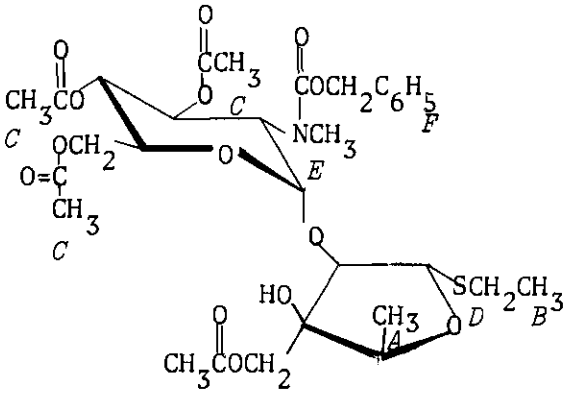


XXII

H	τ	Jcps
A	8.94	6.5
B	8.81	7.1
C	7.40	7.1
D	6.84	
E	2.54	

The mass spectrum of XXII showed peaks at m/e (relative intensity) 456[18, M - SC_2H_5], 310[19, M - dihydrostreptose fragment] and a large number of low mass, high intensity ions.

Acetylation of ethyl N-carbobenzoxy-α-thiodihydrostreptobiosaminide (XXII) by the classical method using pyridine/acetic anhydride gave an oil, XXIII, which after repeated chromatography failed to crystallize. The n.m.r. spectrum was in agreement with the desired structure and absorptions which can be assigned are shown below.



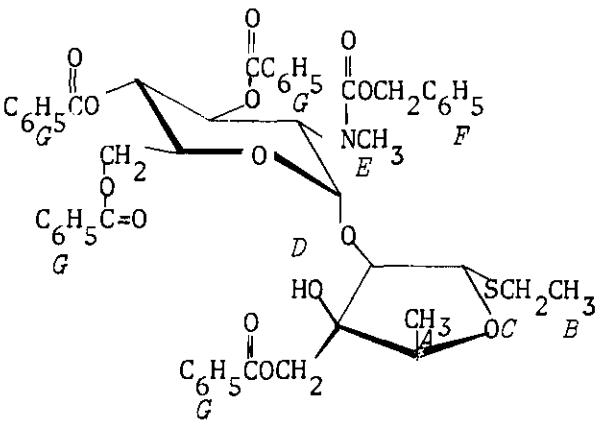
XXIII

H	τ	Jcps
A	8.77	6.5
B	8.71	7.1
C'ε	8.12	
	8.07	
	8.04	
	7.97	
D	7.40	7.1
E	7.06	
F	2.67	

Since it had already been determined that either acetyl or benzoyl groups could be used as O-blocking groups for the synthesis of dihydrostreptomycin, ethyl N-carbobenzoxy-α-thiodihydrostreptobiosaminide, XXII, was benzoylated for the purpose of obtaining a crystalline derivative to use in the glycosidic synthesis.

Original attempts to benzoylate XXII using a large excess of benzoyl chloride (10-20 mole excess) and removal of the excess benzoyl chloride with cold sodium bicarbonate produced large amounts of benzoic anhydride which was impossible to separate from the product. Benzoic anhydride formation was eliminated by using only a slight excess of benzoyl chloride ($\leq$ 1 mole) and removing this excess with a small amount of water. The product, ethyl tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide, XXIV, could easily be obtained crystalline by chromatography over silicic acid. Satisfactory elemental analysis was obtained for the compound, and all spectral properties were in agreement with the assigned

structure. Absorptions in the n.m.r. spectrum that could be assigned are shown below.

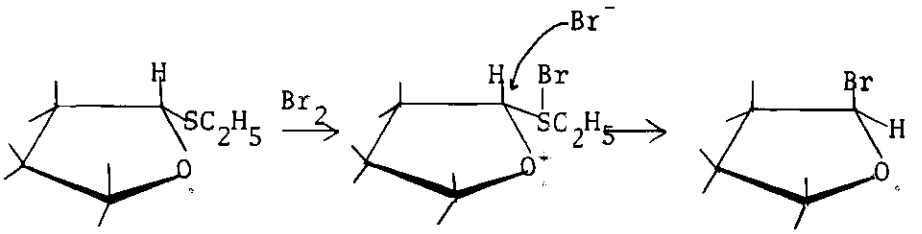


XXIV

H	τ	Jcps
A	8.75	6.5
B	8.88	7.3
C	7.50	7.3
D	7.15	
E	6.93	
F	2.99	
G's	2.98-2.50 (ortho and para)	
	2.20-1.88 (meta)	

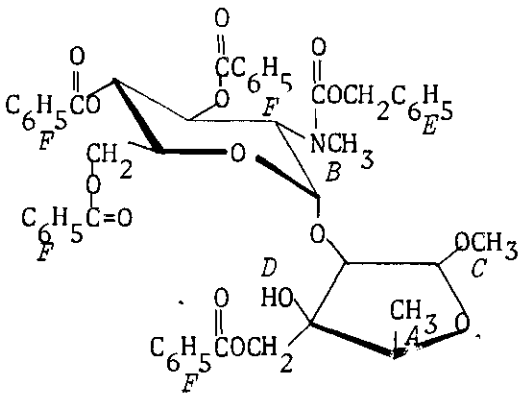
The preparation of compound XXIV completed the synthesis of the dihydrostreptobiosaminide derivative to be used for the attempted reconstitution of dihydrostreptomycin. The next step was to find conditions for the displacement of the ethylthio group.

Thiosugars have been used as precursors of glycosyl halides. The mechanism proposed for the reaction involves formation of a "thiobromonium" ion and then displacement of this complex by a back side attack of a bromide ion, as illustrated below.



Recently, it has been shown that if a bromide ion scavenger, such as silver ion, is placed in the reaction mixture, other nucleophiles such as alcohols will readily displace the "thiobromonium" ion (30). Since the reaction is of the SN_2 type, the configuration of the product depends on the initial configuration of the thio group.

When ethyl tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide (XXIV) was treated with a slight excess of bromine in the presence of methanol and silver carbonate, a crystalline compound could be obtained in 70% yield that was shown by elemental analysis and spectral data to be devoid of the ethylthio group and contain instead a methoxyl group. All data were consistent for the methyl glycoside XXV.



XXV

H	τ	Jcps
A	8.65	6.6
B	6.84	
C	6.65	
D	6.50	
E	2.82	
F's	2.75-2.42 (ortho and para)	
	2.20-1.99 (meta)	

Because the absorption of the anomeric protons in the n.m.r. spectrum were masked by the ring protons of the benzoylated N-methyl-L-glucosamine fragment, a configurational assignment based on the coupling constand of the anomeric proton cannot be made. However, it is possible to make a tentative assignment of the anomeric configuration of the methoxyl group of XXV from the optical rotational values of known streptobiosamine derivatives. Table I lists a number of streptobiosamine derivatives along with the molecular rotation of their respective isomers.

Table 1. Molecular Rotations of Streptobiosamine Derivatives

Streptobiosamine Derivative	Molecular Rotation Isomer		
	α	β	Ref
Methyl tetraacetyl-L-streptobiosaminide	-700[a]	-254°	(13,11)
Ethyl tetraacetyl-L-thiostreptobiosaminide	-1258°	-190°	(15)
Methyl pentaacetyldihydro-L-streptobiosaminide	-659°	-163	(15,13)
Methyl N-acetyl-L-dihydrostreptobiosaminide	-627[c]	-125°	(10)

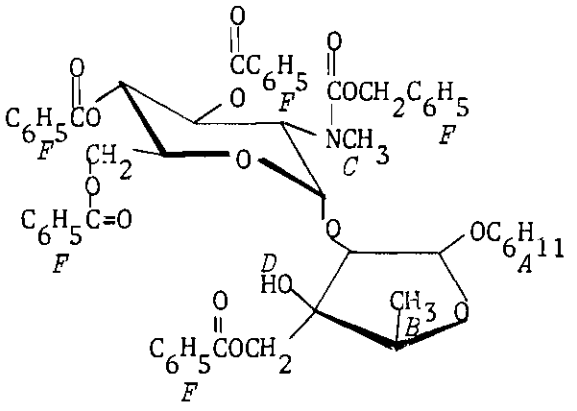
It is important to note the similarity of the rotation of the α and β isomers and that the rotation of the derivatives becomes more positive as the configuration changes from α to β.

It has been noted previously in the chemistry of streptomycin that the molecular rotation values of benzoyl derivatives do not correlate well

with those of acetyl derivatives. Therefore, while the absolute rotational value of compounds XXIV and XXV would not necessarily be expected to be of the same magnitude as those in Table 1, the same shift in rotational values should be observed if a configuration change has occurred at C_1 of the dihydrostreptose fragment.

Ethyl tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide (XXIV) shows a molecular rotation of -308°. The methyl glycoside, XXV, that is prepared from XXIV shows a molecular rotation of 217°. The shift toward a more positive value indicates that methyl tetra-O-benzoyl-N-carbobenzoxythiodihydrostreptobiosaminide (XXV) has the β configuration about C_1 of the dihydrostreptose fragment. The assignment of the β configuration for XXV is in agreement with that expected from the nature of the reaction, i.e., inversion of configuration about the reaction center.

Although the reaction of compound XXIV with methanol demonstrated that the ethylthio group could be displaced, methanol itself because of its relatively small steric requirements as compared to heptabenzoylstreptidine is not a good model. A much better model would be cyclohexanol. When ethyl tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide (XXIV) was reacted with cyclohexanol in the presence of bromine and silver carbonate, the crystalline cyclohexyl glycoside XXVI was obtained in good yield (70%). Elemental analysis and spectral data were in agreement with the proposed structure. The absorptions in the n.m.r. spectrum that can be assigned are shown below.



H	τ	Jcps
A	8.92-8.00	
B	8.70	6.3
C	6.87	
D	6.40	
E	2.99	
F's	2.83-2.67 (ortho para)	
	2.25-1.89 (meta)	

The molecular rotation of compound XXVI, 194°, again suggested the β configuration about C_1 of the dihydrostreptose fragment.

The next step for the reconstitution of dihydrostreptomycin was the reaction of XXIV with heptabenzoylstreptidine, XIV. Although the model reactions indicated that the β-configuration was obtained when ethyl tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide (XXIV) was reacted with a hydroxylic compound, this in itself does not destroy the usefulness of the reaction.

Because of the insolubility of heptabenzoylstreptidine (XIV) in carbon tetrachloride, the solvent used for the preparation of the model compound XXV and XXVI had to be changed to chloroform. Reaction of heptabenzoylstreptidine (XIV) with XXIV followed by chromatography over silicic acid gave a quantitative recovery of heptabenzoylstreptidine (XIV). No attempt was made to recover the dihydrostreptobiosaminide fragment.

The failure to obtain any reconstituted dihydrostreptomycin led to the investigation of a more nucleophilic solvent. Dimethylformamide has

been found to be an excellent solvent for nucleophilic displacement reactions, and it has found increasingly wide application in glycosidic synthesis.

The reaction of ethyl tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide (XXIV) with heptabenzoylstreptidine was repeated using dimethylformamide as the solvent. Even though the reaction mixture was tightly shielded from light, a black suspension of silver was formed that was impossible to remove by either filtration or centrifugation. The product was isolated by removing the dimethylformamide *in vacuo* at 50° and extracting the residue with chloroform. Chromatography of the chloroform extract over silicic acid gave a quantitative recovery of heptabenzoylstreptidine. No attempt was made to isolate the dihydrostreptobiosamine moiety.

The reaction was attempted a third time using dimethoxyethane as solvent. The product after chromatography over silicic acid showed absorptions in the n.m.r. spectrum characteristic of the desired undeca-O-benzoyl-N-carbobenzoxydihydrostreptomycin (XII). Since it was difficult to determine if the product corresponded to the desired material or just a mixture of the two reactants, the protecting groups were removed. Paper chromatography of the resulting material showed only one Weber positive spot corresponding to that of streptidine.

When no product could be obtained between ethyl tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide (XXIV) and heptabenzoylstreptidine (XIV), the possibility that steric hindrance was involved in the lack of reactivity was considered. Since heptaacetylstreptidine (XV) had been reported (25) to react with glycosyl halides to give the corre-

sponding glycoside in poor yield, it was proposed to react XXIV with a large excess of crude heptaacetylstreptidine (XV) in a final attempt to achieve a glycosidic linkage. Peracetyldihydrostreptomycin, IX was cleaved with hydrogen bromide in acetic acid, and the resulting oil was dried to remove any trace of acid. Without further purification the crude heptaacetylstreptidine, XV, was reacted with XXIV using dimethylformamide as the solvent. After removal of the dimethylformamide in vacuo at 50°, the residue was extracted with chloroform. The chloroform extract was chromatographed over silicic acid to yield a material that showed benzoyl absorptions in the n.m.r. spectrum but little acetyl absorption. In order to confirm that no reaction had taken place, the protecting groups were removed in the usual manner to yield a material that was Weber negative. Since the guanidino groups of dihydrostreptomycin gave a strong positive Weber reaction no reaction could have taken place.

CHAPTER IV

RECOMMENDATIONS

Since the main purpose of this research was not achieved, i.e., the glycosidic linkage of dihydrostreptobiosamine with streptidine, a number of recommendations are in order.

The primary objective of any new research should be concerned with conditions and reactants needed to obtain an α-glycosidic linkage between dihydrostreptobiosamine and streptidine. It appears that the thioalkyl group of ethyl thiodihydrostreptobiosaminide is a suitable leaving group for the preparation of alkyl glycosides. Although the configuration obtained seems to be β, this in itself does not destroy the usefulness of the thioalkyl group. Since there appears to be a marked preference for the α-configuration about C_1 of the dihydrostreptose moiety under equilibrium conditions, it is not unreasonable to anticipate that the β configuration, if obtined, could be isomerized to the α-configuration by any one of a number of Lewis acids (27,28,29). Experiments could be conveniently performed using the known methyl and cyclohexyl glycosides of tetra-O-benzoyl-N-carbobenzoxydihydrostreptobiosaminide to determine the feasibility of the isomerization.

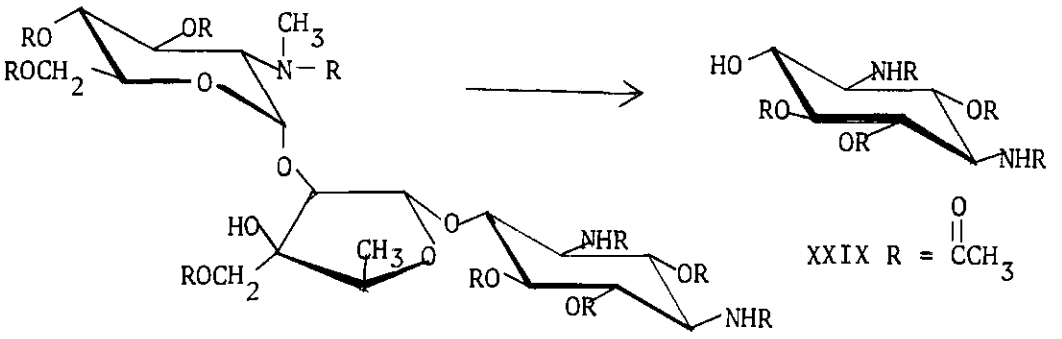
It is also possible to prepare the glycosyl bromide from tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide by reaction of one equivalent of bromine in ether. Very little is known about glycosyl halides of furanoses except that they are less stable than the corresponding

pyranoses (42). The configuration of the halide should be β, and since there are no groups attached to the dihydrostreptose moiety that can participate in a reaction at C_1, displacement of the β-halide with a hydroxylic compound in the manner of Koeing-Knorr should yield an α-glycoside. Preparation of the methyl and cyclohexyl glycosides of the tetra-O-benzoyl-N-carbobenzoxydihydrostreptobiosaminide by this method would also aid in confirming the actual configuration in these glycosides.

Since both heptabenzoyl- and heptaacetylstreptidine failed to react with the thiodihydrostreptiosaminide derivative, it appears that a direct route to the reconstitution of dihydrostreptomycin is no longer possible. An indirect route, but one with a number of advantages, requires the preparation of the streptidine derivative pentaacetylstreptamine.

The preparation of XXIX should be readily accomplished by treatment of the known crystalline decaacetyldideguanyldihydrostreptomycin, XXVII, (44) with hydrogen bromide in acetic acid. Chromatographic separation of the cleavage mixture should yield optically active pentaacetylstreptamine, XXIX and possibly a useful acetylated dihydrostreptiosamine derivative.

Once the pentaacetylstreptamine has been obtained, all efforts should be centered on the glycosidic linkage of it with ethyl tetra-O-benzoyl-N-carbobenzoxy-α-thiodihydrostreptobiosaminide or its glycosyl bromide analog. When conditions are found that give the desired product along with the proper confiruation, it may be convenient to prepare crystalline ethyl pentaacetyl-α-thiodihydrostreptobiosaminide (43) and react this with pentaacetylstreptamine. The product would be XXVII and thus



XXVII R = CCH_3

XXVIII R = H

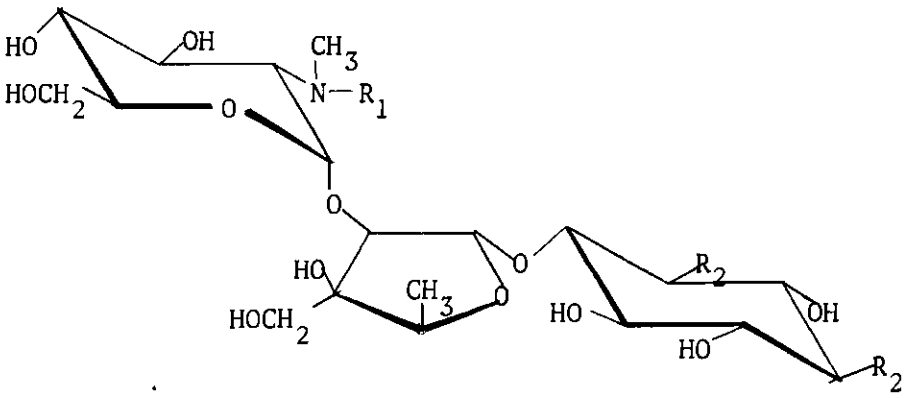
XXIX R = CCH_3

allows all remaining transformation to be performed on naturally derived material. However, since dideguanyldihydrostreptomycin, XXVIII, is stable to boiling base, then, regardless of the protected dihydrostreptobiosamine derivative used, the blocking groups can readily be removed by treatment with base to yield the crystalline dideguanyldihydrostreptomycin, XXVIII (45).

The additional problem presented by this synthetic route is the conversion of dideguanyldihydrostreptomycin to dihydrostreptomycin. Several reagents are available for the conversion of amines to guanidines (46,47,48) and streptamine itself has been converted into streptidine (48,49). However, dideguanyldihydrostreptomycin contains three amino groups, only two of which need to be converted to guanidino groups. Benzylidine derivatives of primary amines are readily formed, and the bis-

benzylidine derivative of streptamine is a known compound (50). Thus, treatment of XXVIII with benzaldehyde under the proper conditions would yield the benzylidine derivative XXX.

The protection of the amino group of the N-methyl-L-glucosamine fragment is dependent on the conditions for removal of the benzylidine groups. If the benzylidine groups can be removed by mild acid hydrolysis or amine exchange in a manner that is non-destructive to the glycosyl



XXX $R_1 = H \quad R_2 = N{=}CHC_6H_5$

XXXI $R_1 = C_6H_3(NO_2)_2 \quad R_2 = N{=}CHC_6H_5$

XXXII $R_1 = C_6H_3(NO_2)_2 \quad R_2 = NH_2$

XXXIII $R_1 = \overset{O}{\overset{\|}{C}}CH_3 \quad R_2 = NH_2$

XXXIV $R_1 = C_6H_3(NO_2)_2 \quad R_2 = \overset{NH}{\overset{\|}{N}}HCNH_2$

XXXV $R_1 = \overset{O}{\overset{\|}{C}}CH_3 \quad R_2 = \overset{NH}{\overset{\|}{N}}HCNH_2$

bonds of XXX, then, the ideal reagent for blocking the remaining amino function is 2,4 dinitrofluorobenzene. The 2,4-dinitrophenyl derivative of N-methyl-L-glucosamine has been prepared (40), and little difficulty should be encountered in preparing this derivative, XXXI. Mild acid hydrolysis should give compound XXXII. However, if it is found necessary to remove the benzylidine groups by reduction, the 2,4-dinitrophenyl derivative, XXXI, cannot be used because the dinitrophenyl group will also be removed by these conditions.

Several reagents are known which allow N-acetylation in the presence of hydroxyl functions (51). Selective N-acetylation of XXX followed by removal of the benzylidine groups would yield the monoacetylated derivative XXXIII. Reaction of either compound XXXII or XXXIII under the conditions that convert streptamine to streptidine should yield 2,4-dinitrophenyldihydrostreptomycin, XXXIV, or N-acetyldihydrostreptomycin, XXXV, respectively.

The 2,4-dinitrophenyl group of XXXIV can easily be removed by reduction to yield dihydrostreptomycin, but N-acetyldihydrostreptomycin has never been successfully converted to dihydrostreptomycin. Recently, a number of new reagents have been reported that hydrolyze amides under mild conditions (52). N-Acetyldihydrostreptomycin is a known compound and attempts to remove the N-acetyl group should be reinvestigated.

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